METHODS FOR TREATING AN ISCHEMIC DISORDER AND IMPROVING STROKE OUTCOME

This application is a continuation-in-part of U.S. Serial No. 09/053,871, filed April 1, 1998 which is a is a continuation-in-part of PCT International Application No. PCT/US97/17229, filed September 25, 1997, which is a continuation-in-part of U.S. Serial No. 08/721,447, filed September 27, 1996 which applications are hereby incorporated by reference in their entireties.

The invention disclosed herein was made with Government support under National Institutes of Health, National Heart, Lung and Blood Institute award HL55397 of the Department of Health and Human Services. This study was also supported in part by the US Public Health Service (R01 HL59488, R01 HL55397, and K08 NS02038). Accordingly, the U.S. Government has certain rights in this invention.

Throughout this application, various publications are referenced following certain Examples and within the Detailed Description of the Invention section. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

Background of the Invention

As described in Colman et al., Editors, Hemostasis and Thrombosis, Third Edition, J.B. Lippincott Company, Philadelphia, 1994, pages 33-36, 62-63 and 94-105, human Factor IX is a 415 amino acid glycoprotein (Mr=57,000, 17% carbohydrate). Factor IX is a proenzyme that has no catalytic activity. During the coagulation cascade, it is cleaved by Factor XIa to produce catalytically active Factor

ANNOTED VERSION OF THE AMENDED PARAGRAPHS

Paragraph on page 110, beginning at line 7:

Carbon monoxide gas, a toxic byproduct of heme catabolism, is involved in long-term potentiation and memory in the central nervous system. However, other physiologic roles for CO production in the brain are unknown. Because heme oxygenase is induced during inflammatory conditions, it was investigated whether endogenous CO production may confer a cerebral protective role in stroke. In a murine model of focal cerebral ischemia, heme oxygenase type I was induced at the mRNA (by Northern blot) and protein levels (by Western blot), localized to the cerebral vascular endothelium in the ischemic hemisphere by in situ hybridization and immunohistochemistry (Figures 26 and 27). Local production of CO by direct measurement was observed in the ischemic zone. In parallel experiments, murine brain endothelial cells exposed to a hypoxic environment demonstrated similar induction of heme oxygenase mRNA, protein and CO generation (Figure 28). To determine whether CO production was incidental to the pathophysiology of stroke, CO production was blocked by tin protoporphyrin administration (confirmed by direct measurement of reduced local CO levels). These animals demonstrated significantly larger infarct volumes, worse neurological outcomes, and increased mortality compared with untreated controls (Figure 24). Furthermore, administration of CO prior to stroke conferred significant cerebral protection (Figure 23). As this protection was not observed in animals treated

with biliverdin, the coincident byproduct of heme catabolism, these data suggest that endogenous CO production per se has a protective role in evolving stroke.

Paragraph on page 111, beginning at line 17:

The current study reports for the first time that the postischemic brain generates enormous quantities of CO. Using a murine model of focal cerebral ischemia in which the middle cerebral artery is occluded by an intraluminal suture, HOI production in the ischemic hemisphere was increased significantly in comparison to the nonischemic hemisphere (Figure 26). Because immunohistochemistry and in situ hybridization localized the source of HOI to endothelial cells within the ischemic hemisphere, an in vitro model of cellular hypoxia was used to confirm the induction of HOI message, protein and activity in murine cerebral microvascular endothelial cells (Figures 29 and 30). Blockade of CO production using tin or zinc protoporphyrin IX was associated with an increase in cerebral infarct volume and mortality, whereas exposing animals to CO immediately prior to ischemia conferred significant dose-dependent cerebral protection within a narrow therapeutic window (Figure 24). Biliverdin administration was without effect in this model. Taken together, these data indicate that ischemic brain tissue produces large amounts of CO, the production of which confers cerebral protection that limits the amount of tissue destroyed during stroke.